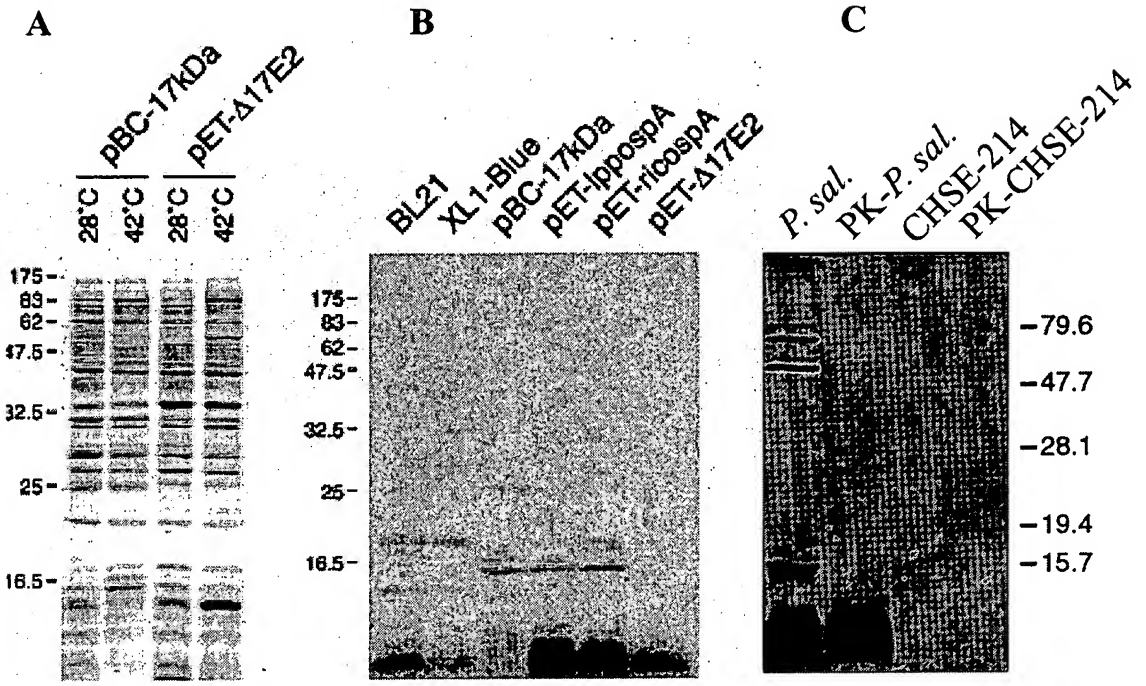
Exhibit A

Figure: Expression, lipidation and Western blot of OspA.



Panel A: SDS PAGE of OspA expressed in *Escherichia coli.* Whole cell lysates of *E. coli* OspA clones were analyzed by SDS-PAGE (15% polyacrylamide). Samples of all OspA constructs at time 0 (28°C) and after 10 hr induction at 42°C were stained with GelCode. Induced production of OspA from constructs pBC-17kDa and pET-Δ17E2 were visible around 16 kDa. Plasmid pBC-17kDa was a clone of the native *ospA* gene from *Piscirickettsia salmonis.* Plasmid pET-Δ17E2 was a clone of synthetic *ospA* gene optimized for *E.* coli expression lacking the signal sequence as would the native *P. salmonis* protein after being processed (signal peptide removed and OspA lipidated) and integrated to the outer membrane. Lane 1: Preinduced sample of *E. coli* harboring plasmid pBC-17kDa and helper plasmid pGP1-2; Lane 2: Induced sample of *E. coli* harboring plasmid pBC-17kDa and helper plasmid pGP1-2; Lane 3: Preinduced sample of *E. coli* harboring plasmids pET-Δ17E2 and helper plasmid pGP1-2; Lane 4: Induced sample of E. coli XL1 Blue harboring plasmids pET-Δ17E2 and helper plasmid pGP1-2. Molecular mass is shown on the right in kDa.

Panel B: [^{14}C]Palmitate incorporation analysis of OspA. [^{14}C]Palmitate-labeled induced cultures of ospA constructs were analyzed by SDS-PAGE (15% polyacrylamide). The first two lanes contain *E. coli* negative controls that were induced under the same conditions as the OspA constructs. Note the [^{14}C]palmitate-labeled product with a relative mobility of < 16 kDa present in induced cultures of pBC-17kDa, pET-lppospA, and pET-ricospA. No labeled products that differed from the *E. coli* BL21 control were observed in the pET-Δ17E2 sample. Molecular mass is on the left in kDa. This shows that OspA is indeed lipidated and signal peptide is being removed.

Panel C: Western blot analysis of P. salmonis. Whole cell lysates and proteinase K digest samples of P. salmonis and CHSE-214 were separated by 12% SDS-PAGE and reacted with 89CR and IPA anti-*P. salmonis* rabbit sera followed by immunochemical detection. Molecular mass is on the left in kDa. Likely the native processed OspA lipoprotein (lacking the signal peptide) was detected as an antigen with a relative mobility of < 16 kDa.

Reverse translation of *ospA* gene

DNA sequence of the native *P. salmonis* gene:

ATGAACAGAGGATGTTTGCAAGGTAGTAGTCTAATTATTATCAGTGTGTTTTTAGTTGGCTGTGC
CCAGAACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCC
AGCTGTTTGGTAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTA
ATTGGTTCTAAAATCGGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGA
AAAGGTAAAAGCAGGGCAAGTGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTTG
AGCCAGTGCGTACTTACCAGCGTTACAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTT
CAGCAAAAGGCGATGATTGCAGGGCAGAAGCAAGAGATTTACGGCACTGCATGCCGGCAACCGGA
TGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Reverse translation of OspA: Molecular Weight 17.660 kDa:

MNRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVAMAIGGAVLGGL
IGSKIGQSMDQQDKIKLNQSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREF
QQKAMIAGQKQEIYGTACRQPDGRWQVISTEK

Reverse translation of the processed OspA (signal peptide removed): Molecular Weight 15.467 kDa:

AQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVAMAIGGAVLGGLIGSKIGQSMDQQDKI
KLNQSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQ
KQEIYGTACRQPDGRWQVISTEK